HUMAN OBESITY SUSCEPTIBILITY GENE AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates generally to the fields of genetics and medicine. The present invention more particularly discloses the identification of a human obesity susceptibility gene, which can be used for the diagnosis, prevention and treatment of obesity and related disorders, as well as for the screening of therapeutically active drugs. The invention more specifically discloses certain alleles of the mitogen activated protein kinase kinase kinase 11 (MAP3K11) gene related to susceptibility to obesity and representing novel targets for therapeutic intervention. The present invention relates to particular mutations in the MAP3K11 gene and expression products, as well as to diagnostic tools and kits based on these mutations. The invention can be used in the diagnosis of predisposition to, detection, prevention and/or treatment of coronary heart disease and metabolic disorders, including hypoalphalipoproteinemia, familial combined hyperlipidemia, insulin resistant syndrome X or multiple metabolic disorder, coronary artery disease, diabetes and dyslipidemic hypertension.

BACKGROUND OF THE INVENTION

Approximately three to eight percent of the total health costs of modern industrialized countries are currently due to the direct costs of obesity (Wolf, 1996). In Germany, the total costs (both direct and indirect) related to obesity and comorbid disorders were estimated at 21 billion German marks in 1995 (Schneider, 1996). By 2030 these costs will rise by 50% even if the prevalence of obesity does not increase further.

Obesity is often defined simply as a condition of abnormal or excessive fat accumulation in adipose tissue, to the extent that health may be impaired. The underlying disease is the process of undesirable positive energy balance and weight gain. An abdominal fat distribution is associated with higher health risks than a gynoid fat distribution.

The body mass index (BMI; kg/m^2) provides the most useful, albeit crude, population-level measure of obesity. It can be used to estimate the prevalence of obesity within a population

and the risks associated with it. However, BMI does not account for body compositon or body fat distribution (WHO, 1998).

Table : Classification of overweight in adults according to BMI (WHO, 1998)

Classification	BMI (kg/m²)	Risk of co-morbidities
Underweight	< 18.5	Low (but risks of other clinical problems increased)
Normal range	18.5 – 24.9	Average
Overweight	> 25	
Pre-obese	25 – 29.9	Increased
Obese class I	30 – 34.9	Moderate
Obese class I	35 – 39.9	Severe
Obese class I	> 40	Very severe

Obesity has also been defined using the 85th and 95th BMI-percentiles as cutoffs for definition of obesity and severe obesity. BMI-percentiles have been calculated within several populations; centiles for the German population based on the German National Nutrition Survey have been available since 1994 (Hebebrand et al., 1994, 1996). Because the WHO classification of the different weight classes can only be applied to adults, it has become costumary to refer to BMI-percentiles for the definition of obesity in children and adolescents.

The recent rise in the prevalence of obesity is an issue of major concern for the health systems of several countries. In the USA the increase in the prevalence of all classes of obesity has been dated to the time period approximately between 1976 and 1990. During this time span, the prevalence of obesity increased by more than one half rising from 14.5% to 22.5%. Similar trends have been observed in other countries in Europe and South America.

Children and adolescents have not been exempt from this trend. Quite to the contrary, the increase in the USA has been substantial. Thus, between the 1960ies and 1990, overweight and obesity increased dramatically in 6 through to 17 year olds. The increments translate into relative increases of 40% using the 85th BMI-centile (calculated in the 1960ies) as a

cutoff and 100% upon use of the 95th centile. In a cross sectional study of German children and adolescents treated as inpatients for extreme obesity between 1985 and 1995, we have reported a significant increase of the mean BMI of almost 2 kg/m^2 over this ten year period. Within this extreme group, the increments were most pronounced in the uppermost BMI ranges.

The mechanisms underlying this increase in the prevalence of obesity are unknown. Environmental factors have commonly been invoked as the underlying cause. Basically, both an increased caloric intake and a reduced level of physical activity have been discussed. In England the increase in obesity rates has been attributed to the latter mechanism. Thus, in this country, the average caloric intake even decreased somewhat within the last two decades, whereas indirect evidence stemming from the increases in hours spent watching television and from the average number of cars per household points to reduced levels of physical activity as the relevant causative factor.

Potentially life-threatening, chronic health problems associated with obesity fall into four main areas: 1) cardiovascular problems, including hypertension, chronic heart disease and stroke, 2) conditions associated with insulin resistance, namely Non-Insulin Dependent Diabetes Mellitus (NIDDM), 3) certain types of cancers, mainly the hormonally related and large-bowel cancers, and 4) gallbladder disease. Other problems associated with obesity include respiratory difficulties, chronic musculo-skeletal problems, skin problems and infertility (WHO, 1998).

The main currently available strategies for treating these disorders include dietary restriction, increments in physical activity, pharmacological and surgical approaches. In adults, long term weight loss is exceptional using conservative interventions. Present pharmacological interventions typically induce a weight loss of between five and fifteen kilograms; if the medication is discontinued, renewed weight gain ensues. Surgical treatments are comparatively successful and are reserved for patients with extreme obesity and/or with serious medical complications.

Recently, a 10 year old massively obese girl, in whom a leptin deficiency mutation had been detected, was treated successfully with recombinant leptin. This is the first individual

who therapeutically profited from the detection of the mutation underlying her morbid obesity.

Several twin studies have been performed to estimate heritability of the BMI, some of which have encompassed over 1000 twin pairs. The results have been very consistent: The intrapair correlations among monozygotic twins were typically between 0.6 and 0.8, independent of age and gender. In one study, the correlations for monozygotic and dizygotic twins were basically the same, independent of whether the twins had been reared apart or together. Heritability of the BMI was estimated at 0.7; non-shared environmental factors explained the remaining 30% of the variance. Surprisingly, shared environmental factors did not explain a substantial proportion of the variance. Both hypercaloric and hypocaloric alimentation lead to similar degrees of weight gain or loss among both members of monozygotic twin pairs, indicating that genetic factors regulate the effect of environmentally induced variation of energy availability on body weight. Metabolic reactions and changes in body fat distribution upon overeating and undereating are also under genetic control (reviewed in Hebebrand et al., 1998).

A large adoption study has revealed that the BMI of adoptees is correlated with that of their biological parents and not with the BMI of the adoptive parents. Depending on the family study, the correlation between the BMI of sibs is between 0.2 and 0.4. Parent-offspring correlations are typically slightly lower. Segregation analyses have repeatedly suggested a major recessive gene effect. Based on these analyses, sample size calculations have been performed based on both concordant and discordant approaches. In contrast to the expectations, the concordant sib-pair approach was superior; a lower number of families were required to achieve the same power.

Family studies based on extremely obese young index patients, either mother or father or both, have a BMI > 90th decile in the vast majority of the families. Based on index patients with a BMI > 95th centile, approximately 20% of the respective families have a sib with a BMI > 90th centile.

In conclusion, it is apparent that environmental factors interact with specific genotypes rendering an individual more or less susceptible to the development of obesity.

Furthermore, despite the fact that major genes have been detected, it is necessary to consider that the spectrum reaches from such major genes to genes with an only minor influence.

The discovery of the leptin gene at the end of 1994 (Zhang et al., 1994) has been followed by a virtual explosion of scientific efforts to uncover the regulatory systems underlying appetite and weight regulation. It is currently the fastest growing biomedical field. This upswing has also resulted in large scaled molecular genetic activities which, due to obvious clinical interest, are basically all related to obesity in humans, rodents and other mammals (Hebebrand et al., 1998).

In this respect, many genes in which mutations lead to the presently known monogenic forms of obesity have been cloned in rodents. Systemic consequences of these mutations are currently being analysed. These models have provided insights into the complex regulatory systems involved in body weight regulation, the best known of which includes leptin and its receptor.

In mice, but also in pigs, over 15 quantitative trait loci (QTL) have been identified that are most likely relevant in weight regulation (Rankinen et al., 2002).

In humans, four exceedingly rare autosomal recessive forms of obesity have been described as of 1997. Mutations in the genes encoding for leptin, leptin receptor, prohormone convertase 1 and pro-opiomelanocortin (POMC) have been shown to cause massive obesity of an early onset type, associated with hyperphagia. Distinct additional clinical (e.g. red hair, primary amenorrhea) and/or endocrinological abnormalities (e.g. markedly altered serum leptin levels, lack of ACTH secretion) pinpointed to the respective candidate genes. Both the monogenic animal models and the human monogenic forms have led to new insights into the complex system underlying body weight regulation.

Very recently, the first autosomal dominant form of obesity was described in humans. Two different mutations within the melanocortin-4 receptor gene (*MC4R*) were observed to lead to extreme obesity in probands heterozygous for these variants. In contrast to the aforementioned findings, these mutations do not implicate readily obvious phenotypic abnormalities other than extreme obesity (Vaisse et al., 1998; Yeo et al., 1998).

Interestingly, both groups detected the mutations by systematic screens in relatively small study groups (n=63 and n=43).

Hinney et al. (1999) screened the *MC4R* in a total of 492 obese children and adolescents. All in all, four individuals with two different mutations leading to haplo-insufficiency were detected. One was identical to that previously observed by Yeo et al. (1998). The other mutation, which was detected in three individuals, induced a stop mutation in the extracellular domain of the receptor. Approximately one percent of extremely obese individuals harbour haplo-insufficiency mutations in the *MC4R*. In addition to the two forms of haplo-insufficiency, Hinney et al. (1999) also detected additional mutations leading to both conservative and non-conservative amino acid exchanges. Interestingly, these mutations were mainly observed in the obese study group. The functional implications of these mutations are currently unknown.

The identification of individuals with *MC4R* mutations is interesting in the light of possible pharmacological interventions. Thus, intranasal application of adrenocorticotropin4-10 ($ACTH_{4-10}$), representing a core sequence of all melanocortins, resulted in reduced weight, body fat mass and plasma leptin concentrations in healthy controls. The question arises as to how mutation carriers would react to this treatment, which could theoretically counterbalance their reduced receptor density.

The involvement of specific genes in weight regulation is further substantiated by data obtained from transgenic mice. For example, MC4R deficient mice develop early onset obesity (Huszar et al., 1997).

Different groups are conducting genome scans related to obesity or dependent phenotypes (BMI, leptin levels, fat mass, etc.). This approach appears very promising, because it is both systematic and model free. In addition, it has already been shown to be exceptionally successful. Thus, positive linkage results have been obtained even by analysing comparatively small study groups. More important, some findings have already been replicated. Each of the following regions has been identified by at least two independent groups: chromosome 1q32, chromosome 2p21, chromosome 10 and chromosome 20q13 (Rankinen et al., 2002).

SUMMARY OF THE INVENTION

The present invention now discloses the identification of a human obesity susceptibility gene, which can be used for the diagnosis, prevention and treatment of obesity and related disorders, as well as for the screening of therapeutically active drugs.

The invention can be used in the diagnosis of predisposition to, detection, prevention and/or treatment of obesity, coronary heart disease and metabolic disorders, including hypoalphalipoproteinemia, familial combined hyperlipidemia, insulin resistant syndrome X or multiple metabolic disorder, coronary artery disease, diabetes and dyslipidemic hypertension, the method comprising detecting in a sample from the subject the presence of an alteration in the MAP3K11 gene or polypeptide, the presence of said alteration being indicative of the presence or predisposition to obesity or associated disorders.

The invention also resides in methods of treating obesity and/or associated disorders in a subject through a modulation of MAP3K11 expression or activity. Such treatments use, for instance, MAP3K11 polypeptides, MAP3K11 DNA sequences (including antisense sequences and RNAi directed at the MAP3K11 gene locus), anti-MAP3K11 antibodies or drugs that modulate MAP3K11 expression or activity.

The invention also relates to methods of treating individuals who carry deleterious alleles of the MAP3K11 gene, including pre-symptomatic treatment or combined therapy, such as through gene therapy, protein replacement therapy or through the administration of MAP3K11 protein mimetics and/or inhibitors.

A further aspect of this invention resides in the screening of drugs for therapy of obesity or associated disorder, based on the modulation of or binding to an allele of MAP3K11 gene associated with obesity or associated disorder or gene product thereof.

The invention further relates to the screening of alteration(s) associated with obesity or associated disorder in the MAP3K11 gene locus in patients. Such screenings are useful for

diagnosing the presence, risk or predisposition to obesity and associated disorders, and/or for assessing the efficacy of a treatment of such disorders.

The invention also resides in particular products such as primers, probes, oligonucleotides and substrates or supports to which said products are immobilized, which are designed to specifically detect or amplify an altered MAP3K11 gene or gene product. The invention also concerns the use of primers, probes, oligonucleotides and substrates or supports to which said products are immobilized, for the detection of an altered MAP3K11 gene or gene product.

A further aspect of this invention includes antibodies specific of MAP3K11 polypeptide fragments and derivatives of such antibodies, hybridomas secreting such antibodies, and diagnostic kits comprising those antibodies. More preferably, said antibodies are specific to a MAP3K11 polypeptide or a fragment thereof comprising an alteration, said alteration modifying the activity of MAP3K11.

The invention also concerns a MAP3K11 gene or a fragment thereof comprising an alteration, said alteration modifying the activity of MAP3K11. The invention further concerns a MAP3K11 polypeptide or a fragment thereof comprising an alteration, said alteration modifying the activity of MAP3K11.

LEGEND TO THE FIGURES

Figure 1 : Graphical presentation of results for human chromosome 11 from a genome wide microsatellite scan for regions linked to obesity. Results obtained for microsatellites in the region of chromosome 11q13.1 show evidence for linkage in the region of the MAP kinase. Non-parametric linkage analysis showed a maximum LOD-score for markers D11S903, D11S1313 and D11S1883 (LOD= 1.48).

Figure 2 : High density mapping using Genomic Hybrid Identity Profiling (GenomeHIP). Graphical presentation of the linkage peak on chromosome 11q13. The curves depict the linkage results for the GenomeHIP procedure in the region. The dotted lines correspond to

the Lander & Krygliak thresholds for suggestive evidence and evidence for linkage respectively.

A total of 46 BAC clones on human chromosome 11q ranging from position cen-45451827 to 84002801-q-ter were tested for linkage using GenomeHIP. Each point on the x-axis corresponds to a clone. Several clones are indicated by their library name for better orientation (e.g. RP11-193F22). The two horizontal lines at $3*10^{-4}$ and $2*10^{-5}$ for the p-values correspond to the significance levels for significant and suggestive linkage proposed by Krygliak and Lander for whole genome screens. Significant evidence for linkage was calculated for clone RP11-9K14 ($p< 2.5 \times 10^{-5}$).

DETAILED DESCRIPTION OF THE INVENTION

The present invention discloses the identification of MAP3K11 as a human obesity susceptibility gene. Various nucleic acid samples from 89 families with obesity were submitted to a particular GenomeHIP process. This process led to the identification of particular identical-by-descent fragments in said populations that are altered in obese subjects. By screening of the IBD fragments, we identified the mitogen activated protein kinase kinase kinase on chromosome 11q13.1 (MAP3K11) gene as a candidate for obesity and related phenotypes. This gene is indeed present in the critical interval and expresses a functional phenotype consistent with a genetic regulation of obesity.

The present invention thus proposes to use MAP3K11 gene and corresponding expression products for the diagnosis, prevention and treatment of obesity and associated disorders, as well as for the screening of therapeutically active drugs.

DEFINITIONS

Obesity and metabolic disorders: Obesity shall be construed as any condition of abnormal or excessive fat accumulation in adipose tissue, to the extent that health may be impaired. Associated disorders, diseases or pathologies include, more specifically, any metabolic disorders, including hypo-alphalipoproteinemia, familial combined hyperlipidemia, insulin resistant syndrome X or multiple metabolic disorder, coronary artery disease, diabetes

mellitus and dyslipidemic hypertension. The invention may be used in various subjects, particularly human, including adults, children and at the prenatal stage.

Within the context of this invention, the MAP3K11 gene locus designates all MAP3K11 sequences or products in a cell or organism, including MAP3K11 coding sequences, MAP3K11 non-coding sequences (e.g., introns), MAP3K11 regulatory sequences controlling transcription and/or translation (e.g., promoter, enhancer, terminator, etc.), as well as all corresponding expression products, such as MAP3K11 RNAs (e.g., mRNAs) and MAP3K11 polypeptides (e.g., a pre-protein and a mature protein).

As used in the present application, the term "MAP3K11 gene" designates the human mitogen activated protein kinase kinase kinase gene on human chromosome 11, as well as variants, analogs and fragments thereof, including alleles thereof (e.g., germline mutations) which are related to susceptibility to obesity and metabolic disorders. The MAP3K11 gene may also be referred to as MLK3, PTK1, SPRK, MLK-3 or MGC17114.

The term "gene" shall be construed to include any type of coding nucleic acid, including genomic DNA (gDNA), complementary DNA (cDNA), synthetic or semi-synthetic DNA, as well as any form of corresponding RNA. The term gene particularly includes recombinant nucleic acids encoding MAP3K11, i.e., any non naturally occurring nucleic acid molecule created artificially, e.g., by assembling, cutting, ligating or amplifying sequences. A MAP3K11 gene is typically double-stranded, although other forms may be contemplated, such as single-stranded. MAP3K11 genes may be obtained from various sources and according to various techniques known in the art, such as by screening DNA libraries or by amplification from various natural sources. Recombinant nucleic acids may be prepared by conventional techniques, including chemical synthesis, genetic engineering, enzymatic techniques, or a combination thereof. Suitable MAP3K11 gene sequences may be found on gene banks, such as Unigene Cluster for MAP3K11 (Hs. NM_002419). A particular example of a MAP3K11 gene comprises SEQ ID No: 1.

The term "MAP3K11 gene" includes any variant, fragment or analog of SEQ ID No:1 or of any coding sequence as identified above. Such variants include, for instance, naturally-occurring variants due to allelic variations between individuals (e.g., polymorphisms), mutated alleles related to obesity, alternative splicing forms, etc. The term variant also

includes MAP3K11 gene sequences from other sources or organisms. Variants are preferably substantially homologous to SEQ ID No:1, i.e., exhibit a nucleotide sequence identity of at least about 65%, typically at least about 75%, preferably at least about 85%, more preferably at least about 95% with SEQ ID No:1. Variants and analogs of a MAP3K11 gene also include nucleic acid sequences, which hybridize to a sequence as defined above (or a complementary strand thereof) under stringent hybridization conditions. Typical stringent hybridisation conditions include temperatures above 30° C, preferably above 35°C, more preferably in excess of 42°C, and/or salinity of less than about 500 mM, preferably less than 200 mM. Hybridization conditions may be adjusted by the skilled person by modifying the temperature, salinity and/or the concentration of other reagents such as SDS, SSC, etc.

A fragment of a MAP3K11 gene designates any portion of at least about 8 consecutive nucleotides of a sequence as disclosed above, preferably at least about 15, more preferably at least about 20 nucleotides, further preferably of at least 30 nucleotides. Fragments include all possible nucleotide length between 8 and 100 nucleotides, preferably between 15 and 100, more preferably between 20 and 100.

A MAP3K11 polypeptide designates any protein or polypeptide encoded by a MAP3K11 gene as disclosed above. The term "polypeptide" refers to any molecule comprising a stretch of amino acids. This term includes molecules of various length, such as peptides and proteins. The polypeptide may be modified, such as by glycosylations and/or acetylations and/or chemical reaction or coupling, and may contain one or several non-natural or synthetic amino acids. A specific example of a MAP3K11 polypeptide comprises all or part of SEQ ID No:2 or a variant thereof.

DIAGNOSIS

The invention now provides diagnosis methods based on a monitoring of the MAP3K11 gene locus in a subject. Within the context of the present invention, the term 'diagnosis" includes the detection, monitoring, dosing, comparison, etc., at various stages, including early, pre-symptomatic stages, and late stages, in adults, children and pre-birth. Diagnosis typically includes the prognosis, the assessment of a predisposition or risk of development,

the characterization of a subject to define most appropriate treatment (pharmaco-genetics), etc.

A particular object of this invention resides in a method of detecting the presence of or predisposition to obesity or an associated disorder in a subject, the method comprising detecting in a sample from the subject the presence of an alteration in the MAP3K11 gene locus in said sample. The presence of said alteration is indicative of the presence or predisposition to obesity or an associated disorder. Optionally, said method comprises a previous step of providing a sample from a subject. Preferably, the presence of an alteration in the MAP3K11 gene locus in said sample is detected through the genotyping of a sample.

Another particular object of this invention resides in a method of assessing the response of a subject to a treatment of obesity or an associated disorder, the method comprising detecting in a sample from the subject the presence of an alteration in the MAP3K11 gene locus in said sample. The presence of said alteration is indicative of a particular response to said treatment. Preferably, the presence of an alteration in the MAP3K11 gene locus in said sample is detected through the genotyping of a sample.

The alteration may be determined at the level of the MAP3K11 gDNA, RNA or polypeptide. Optionally, the detection is performed by sequencing all or part of the MAP3K11 gene or by selective hybridisation or amplification of all or part of the MAP3K11 gene. More preferably a MAP3K11 gene specific amplification is carried out before the alteration identification step.

An alteration in the MAP3K11 gene locus may be any form of mutation(s), deletion(s), rearrangement(s) and/or insertions in the coding and/or non-coding region of the locus, alone or in various combination(s). Mutations more specifically include point mutations. Deletions may encompass any region of two or more residues in a coding or non-coding portion of the gene locus, such as from two residues up to the entire gene or locus. Typical deletions affect smaller regions, such as domains (introns) or repeated sequences or fragments of less than about 50 consecutive base pairs, although larger deletions may occur as well. Insertions may encompass the addition of one or several residues in a coding or non-coding portion of the gene locus. Insertions may typically comprise an addition of

between 1 and 50 base pairs in the gene locus. Rearrangement includes inversion of sequences. The MAP3K11 gene locus alteration may result in the creation of stop codons, frameshift mutations, amino acid substitutions, particular RNA splicing or processing, product instability, truncated polypeptide production, etc. The alteration may result in the production of a MAP3K11 polypeptide with altered function, stability, targeting or structure. The alteration may also cause a reduction in protein expression or, alternatively, an increase in said production.

In a first variant, the method of the present invention comprises detecting the presence of an altered MAP3K11 gene sequence. This can be performed by sequencing all or part of the MAP3K11 gene, polypeptide or RNA, by selective hybridisation or by selective amplification, for instance.

In another variant, the method comprises detecting the presence of an altered MAP3K11 RNA expression. Altered RNA expression includes the presence of an altered RNA sequence, the presence of an altered RNA splicing or processing, the presence of an altered quantity of RNA, etc. These may be detected by various techniques known in the art, including by sequencing all or part of the MAP3K11 RNA or by selective hybridisation or selective amplification of all or part of said RNA, for instance.

In a further variant, the method comprises detecting the presence of an altered MAP3K11 polypeptide expression. Altered MAP3K11 polypeptide expression includes the presence of an altered polypeptide sequence, the presence of an altered quantity of MAP3K11 polypeptide, the presence of an altered tissue distribution, etc. These may be detected by various techniques known in the art, including by sequencing and/or binding to specific ligands (such as antibodies), for instance.

As indicated above, various techniques known in the art may be used to detect or quantify altered MAP3K11 gene or RNA expression or sequence, including sequencing, hybridisation, amplification and/or binding to specific ligands (such as antibodies). Other suitable methods include allele-specific oligonucleotide (ASO), allele-specific amplification, Southern blot (for DNAs), Northern blot (for RNAs), single-stranded conformation analysis (SSCA), PFGE, fluorescent in situ hybridization (FISH), gel migration, clamped denaturing gel electrophoresis, heteroduplex analysis, RNase

protection, chemical mismatch cleavage, ELISA, radio-immunoassays (RIA) and immuno-enzymatic assays (IEMA).

Some of these approaches (e.g., SSCA and CGGE) are based on a change in electrophoretic mobility of the nucleic acids, as a result of the presence of an altered sequence. According to these techniques, the altered sequence is visualized by a shift in mobility on gels. The fragments may then be sequenced to confirm the alteration.

Some others are based on specific hybridisation between nucleic acids from the subject and a probe specific for wild-type or altered MAP3K11 gene or RNA. The probe may be in suspension or immobilized on a substrate. The probe is typically labelled to facilitate detection of hybrids.

Some of these approaches are particularly suited for assessing a polypeptide sequence or expression level, such as Northern blot, ELISA and RIA. These latter require the use of a ligand specific for the polypeptide, more preferably of a specific antibody.

In a particular, preferred, embodiment, the method comprises detecting the presence of an altered MAP3K11 gene expression profile in a sample from the subject. As indicated above, this can be accomplished more preferably by sequencing, selective hybridisation and/or selective amplification of nucleic acids present in said sample.

Sequencing

Sequencing can be carried out using techniques well known in the art, using automatic sequencers. The sequencing may be performed on the complete MAP3K11 gene or, more preferably, on specific domains thereof, typically those known or suspected to carry deleterious mutations or other alterations.

Amplification

Amplification is based on the formation of specific hybrids between complementary nucleic acid sequences that serve to initiate nucleic acid reproduction.

Amplification may be performed according to various techniques known in the art, such as by polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA) and nucleic acid sequence based amplification (NASBA). These techniques can be performed using commercially available reagents and protocols. Preferred techniques use allele-specific PCR or PCR-SSCP. Amplification usually requires the use of specific nucleic acid primers, to initiate the reaction.

Nucleic acid primers useful for amplifying sequences from the MAP3K11 gene or locus are able to specifically hybridize with a portion of the MAP3K11 gene locus that flank a target region of said locus, said target region being altered in certain subjects having obesity or associated disorders.

Primers that can be used to amplify MAP3K11 target region may be designed based on the sequence of SEQ ID No: 1.

The invention also relates to a nucleic acid primer, said primer being complementary to and hybridizing specifically to a portion of a MAP3K11 coding sequence (e.g., gene or RNA) altered in certain subjects having obesity or associated disorders. In this regard, particular primers of this invention are specific for altered sequences in a MAP3K11 gene or RNA. By using such primers, the detection of an amplification product indicates the presence of an alteration in the MAP3K11 gene locus. In contrast, the absence of amplification product indicates that the specific alteration is not present in the sample.

Typical primers of this invention are single-stranded nucleic acid molecules of about 5 to 60 nucleotides in length, more preferably of about 8 to about 25 nucleotides in length. The sequence can be derived directly from the sequence of the MAP3K11 gene locus. Perfect complementarity is preferred, to ensure high specificity. However, certain mismatch may be tolerated.

The invention also concerns the use of a nucleic acid primer or a pair of nucleic acid primers as described above in a method of detecting the presence of or predisposition to obesity or an associated disorder in a subject or in a method of assessing the response of a subject to a treatment of obesity or an associated disorder.

Selective hybridization

Hybridization detection methods are based on the formation of specific hybrids between complementary nucleic acid sequences that serve to detect nucleic acid sequence alteration(s).

A particular detection technique involves the use of a nucleic acid probe specific for wild-type or altered MAP3K11 gene or RNA, followed by the detection of the presence of a hybrid. The probe may be in suspension or immobilized on a substrate or support (as in nucleic acid array or chips technologies). The probe is typically labelled to facilitate detection of hybrids.

In this regard, a particular embodiment of this invention comprises contacting the sample from the subject with a nucleic acid probe specific for an altered MAP3K11 gene locus, and assessing the formation of an hybrid. In a particular, preferred embodiment, the method comprises contacting simultaneously the sample with a set of probes that are specific, respectively, for wild type MAP3K11 gene locus and for various altered forms thereof. In this embodiment, it is possible to detect directly the presence of various forms of alterations in the MAP3K11 gene locus in the sample. Also, various samples from various subjects may be treated in parallel.

Within the context of this invention, a probe refers to a polynucleotide sequence which is complementary to and capable of specific hybridisation with a (target portion of a) MAP3K11 gene or RNA, and which is suitable for detecting polynucleotide polymorphisms associated with MAP3K11 alleles which predispose to or are associated with obesity or metabolic disorders. Probes are preferably perfectly complementary to the MAP3K11 gene, RNA, or target portion thereof. Probes typically comprise single-stranded nucleic acids of between 8 to 1000 nucleotides in length, for instance of between 10 and 800, more preferably of between 15 and 700, typically of between 20 and 500. It should be understood that longer probes may be used as well. A preferred probe of this invention is a single stranded nucleic acid molecule of between 8 to 500 nucleotides in length, which can specifically hybridise to a region of a MAP3K11 gene or RNA that carries an alteration.

A specific embodiment of this invention is a nucleic acid probe specific for an altered (e.g., a mutated) MAP3K11 gene or RNA, i.e., a nucleic acid probe that specifically hybridises to said altered MAP3K11 gene or RNA and essentially does not hybridise to a MAP3K11 gene or RNA lacking said alteration. Specificity indicates that hybridisation to the target sequence generates a specific signal which can be distinguished from the signal generated through non-specific hybridisation. Perfectly complementary sequences are preferred to design probes according to this invention. It should be understood, however, that certain mismatch may be tolerated, as long as the specific signal may be distinguished from non-specific hybridisation.

The sequence of the probes can be derived from the sequences of the MAP3K11 gene and RNA as provided in the present application. Nucleotide substitutions may be performed, as well as chemical modifications of the probe. Such chemical modifications may be accomplished to increase the stability of hybrids (e.g., intercalating groups) or to label the probe. Typical examples of labels include, without limitation, radioactivity, fluorescence, luminescence, enzymatic labelling, etc.

The invention also concerns the use of a nucleic acid probe as described above in a method of detecting the presence of or predisposition to obesity or an associated disorder in a subject or in a method of assessing the response of a subject to a treatment of obesity or an associated disorder.

Specific Ligand Binding

As indicated above, alteration in the MAP3K11 gene locus may also be detected by screening for alteration(s) in MAP3K11 polypeptide sequence or expression levels. In this regard, a specific embodiment of this invention comprises contacting the sample with a ligand specific for a MAP3K11 polypeptide and determining the formation of a complex.

Different types of ligands may be used, such as specific antibodies. In a specific embodiment, the sample is contacted with an antibody specific for a MAP3K11 polypeptide and the formation of an immune complex is determined. Various methods for detecting an immune complex can be used, such as ELISA, radio-immunoassays (RIA) and immuno-enzymatic assays (IEMA).

Within the context of this invention, an antibody designates a polyclonal antibody, a monoclonal antibody, as well as fragments or derivatives thereof having substantially the same antigen specificity. Fragments include Fab, Fab'2, CDR regions, etc. Derivatives include single-chain antibodies, humanized antibodies, poly-functional antibodies, etc.

An antibody specific for a MAP3K11 polypeptide designates an antibody that selectively binds a MAP3K11 polypeptide, i.e., an antibody raised against a MAP3K11 polypeptide or an epitope-containing fragment thereof. Although non-specific binding towards other antigens may occur, binding to the target MAP3K11 polypeptide occurs with a higher affinity and can be reliably discriminated from non-specific binding.

In a specific embodiment, the method comprises contacting a sample from the subject with (a support coated with) an antibody specific for an altered form of a MAP3K11 polypeptide, and determining the presence of an immune complex. In a particular embodiment, the sample may be contacted simultaneously, or in parallel, or sequentially, with various (supports coated with) antibodies specific for different forms of a MAP3K11 polypeptide, such as a wild-type and various altered forms thereof.

The invention also concerns the use of a ligand, preferably an antibody, a fragment or a derivative thereof as described above, in a method of detecting the presence of or predisposition to obesity or associated disorders in a subject or in a method of assessing the response of a subject to a treatment of obesity or associated disorders.

The invention also relates to a diagnostic kit comprising products and reagents for detecting in a sample from a subject the presence of an alteration in the MAP3K11 gene or polypeptide, in the MAP3K11 gene or polypeptide expression, and/or in MAP3K11 activity. Said diagnostic kit according to the present invention comprises any primer, any pair of primers, any nucleic acid probe and/or any ligand, preferably antibody, described in the present invention. Said diagnostic kit according to the present invention can further comprise reagents and/or protocols for performing a hybridization, amplification or antigen-antibody immune reaction.

The diagnosis methods can be performed in vitro, ex vivo or in vivo, preferably in vitro or ex vivo. They use a sample from the subject, to assess the status of the MAP3K11 gene locus. The sample may be any biological sample derived from a subject, which contains nucleic acids or polypeptides. Examples of such samples include fluids, tissues, cell samples, organs, biopsies, etc. Most preferred samples are blood, plasma, saliva, urine, seminal fluid, etc. Pre-natal diagnosis may also be performed by testing foetal cells or placental cells, for instance The sample may be collected according to conventional techniques and used directly for diagnosis or stored. The sample may be treated prior to performing the method, in order to render or improve availability of nucleic acids or polypeptides for testing. Treatments include, for instant, lysis (e.g., mechanical, physical, chemical, etc.), centrifugation, etc. Also, the nucleic acids and/or polypeptides may be pre-purified or enriched by conventional techniques, and/or reduced in complexity. Nucleic acids and polypeptides may also be treated with enzymes or other chemical or physical treatments to produce fragments thereof. Considering the high sensitivity of the claimed methods, very few amounts of sample are sufficient to perform the assay.

As indicated, the sample is preferably contacted with reagents such as probes, primers or ligands in order to assess the presence of an altered MAP3K11 gene locus. Contacting may be performed in any suitable device, such as a plate, tube, well, glass, etc. In specific embodiments, the contacting is performed on a substrate coated with the reagent, such as a nucleic acid array or a specific ligand array. The substrate may be a solid or semi-solid substrate such as any support comprising glass, plastic, nylon, paper, metal, polymers and the like. The substrate may be of various forms and sizes, such as a slide, a membrane, a bead, a column, a gel, etc. The contacting may be made under any condition suitable for a complex to be formed between the reagent and the nucleic acids or polypeptides of the sample.

The finding of an altered MAP3K11 polypeptide, RNA or DNA in the sample is indicative of the presence of an altered MAP3K11 gene locus in the subject, which can be correlated to the presence, predisposition or stage of progression of obesity or metabolic disorders. For example, an individual having a germline MAP3K11 mutation has an increased risk of developing obesity or metabolic disorders. The determination of the presence of an altered MAP3K11 gene locus in a subject also allows the design of appropriate therapeutic

intervention, which is more effective and customized. Also, this determination at the pre-symptomatic level allows a preventive regimen to be applied.

GENE, VECTORS, RECOMBINANT CELLS AND POLYPEPTIDES

A further aspect of this invention resides in novel products for use in diagnosis, therapy or screening. These products comprise nucleic acid molecules encoding a MAP3K11 polypeptide or a fragment thereof, vectors comprising the same, recombinant host cells and expressed polypeptides.

More particularly, the invention concerns an altered or mutated MAP3K11 gene or a fragment thereof comprising said alteration or mutation. The invention also concerns nucleic acid molecules encoding an altered or mutated MAP3K11 polypeptide or a fragment thereof comprising said alteration or mutation. Said alteration or mutation modifies the MAP3K11 activity. The modified activity can be increased or decreased. The invention further concerns a vector comprising an altered or mutated MAP3K11 gene or a fragment thereof comprising said alteration or mutation or a nucleic acid molecule encoding an altered or mutated MAP3K11 polypeptide or a fragment thereof comprising said alteration or mutation, recombinant host cells and expressed polypeptides.

A further object of this invention is a vector comprising a nucleic acid encoding a MAP3K11 polypeptide according to the present invention. The vector may be a cloning vector or, more preferably, an expression vector, i.e., a vector comprising regulatory sequences causing expression of a MAP3K11 polypeptide from said vector in a competent host cell.

These vectors can be used to express a MAP3K11 polypeptide in vitro, ex vivo or in vivo, to create transgenic or "Knock Out" non-human animals, to amplify the nucleic acids, to express antisense RNAs, etc.

The vectors of this invention typically comprise a MAP3K11 coding sequence according to the present invention operably linked to regulatory sequences, e.g., a promoter, a polyA, etc. The term "operably linked" indicates that the coding and regulatory sequences are

functionally associated so that the regulatory sequences cause expression (e.g., transcription) of the coding sequences. The vectors may further comprise one or several origins of replication and/or selectable markers. The promoter region may be homologous or heterologous with respect to the coding sequence, and provide for ubiquitous, constitutive, regulated and/or tissue specific expression, in any appropriate host cell, including for in vivo use. Examples of promoters include bacterial promoters (T7, pTAC, Trp promoter, etc.), viral promoters (LTR, TK, CMV-IE, etc.), mammalian gene promoters (albumin, PGK, etc), and the like.

The vector may be a plasmid, a virus, a cosmid, a phage, a BAC, a YAC, etc. Plasmid vectors may be prepared from commercially available vectors such as pBluescript, pUC, pBR, etc. Viral vectors may be produced from baculoviruses, retroviruses, adenoviruses, AAVs, etc., according to recombinant DNA techniques known in the art.

In this regard, a particular object of this invention resides in a recombinant virus encoding a MAP3K11 polypeptide as defined above. The recombinant virus is preferably replication-defective, even more preferably selected from E1- and/or E4-defective adenoviruses, Gag-, pol- and/or env-defective retroviruses and Rep- and/or Cap-defective AAVs. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include PA317 cells, PsiCRIP cells, GPenv+ cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in WO95/14785, WO96/22378, US5,882,877, US6,013,516, US4,861,719, US5,278,056 and WO94/19478.

A further object of the present invention resides in a recombinant host cell comprising a recombinant MAP3K11 gene or a vector as defined above. Suitable host cells include, without limitation, prokaryotic cells (such as bacteria) and eukaryotic cells (such as yeast cells, mammalian cells, insect cells, plant cells, etc.). Specific examples include E.coli, Kluyveromyces or Saccharomyces yeasts, mammalian cell lines (e.g., Vero cells, CHO cells, 3T3 cells, COS cells, etc.) as well as primary or established mammalian cell cultures (e.g., produced from fibroblasts, embryonic cells, epithelial cells, nervous cells, adipocytes, etc.).

The present invention also relates to a method for producing a recombinant host cell expressing a MAP3K11 polypeptide according to the present invention, said method comprising (i) introducing in vitro or ex vivo into a competent host cell a recombinant nucleic acid or a vector as described above, (ii) culturing in vitro or ex vivo the recombinant host cells obtained and (iii), optionally, selecting the cells which express the MAP3K11 polypeptide.

Such recombinant host cells can be used for the production of MAP3K11 polypeptides, as well as for screening of active molecules, as described below. Such cells may also be used as a model system to study obesity and metabolic disorders. These cells can be maintained in suitable culture media, such as DMEM, RPMI, HAM, etc., in any appropriate culture device (plate, flask, dish, tube, pouch, etc.).

DRUG SCREENING

The present invention also provides novel targets and methods for the screening of drug candidates or leads. The methods include binding assays and/or functional assays, and may be performed in vitro, in cell systems, in animals, etc.

A particular object of this invention resides in a method of selecting biologically active compounds, said method comprising contacting in vitro a test compound with a MAP3K11 gene or polypeptide according to the present invention and determining the ability of said test compound to bind said MAP3K11 gene or polypeptide. Binding to said gene or polypeptide provides an indication as to the ability of the compound to modulate the activity of said target, and thus to affect a pathway leading to obesity or metabolic disorders in a subject. In a preferred embodiment, the method comprises contacting in vitro a test compound with a MAP3K11 polypeptide or a fragment thereof according to the present invention and determining the ability of said test compound to bind said MAP3K11 polypeptide or fragment. The fragment preferably comprises a binding site of the MAP3K11 polypeptide. Preferably, said MAP3K11 gene or polypeptide or a fragment thereof is an altered or mutated MAP3K11 gene or polypeptide or a fragment thereof comprising the alteration or mutation.

A particular object of this invention resides in a method of selecting compounds active on obesity and associated disorders, said method comprising contacting in vitro a test compound with a MAP3K11 polypeptide according to the present invention or binding site-containing fragment thereof and determining the ability of said test compound to bind said MAP3K11 polypeptide or fragment thereof. Preferably, said MAP3K11 polypeptide or a fragment thereof is an altered or mutated MAP3K11 polypeptide or a fragment thereof comprising the alteration or mutation.

In a further particular embodiment, the method comprises contacting a recombinant host cell expressing a MAP3K11 polypeptide according to the present invention with a test compound, and determining the ability of said test compound to bind said MAP3K11 and to modulate the activity of MAP3K11 polypeptide. Preferably, said MAP3K11 polypeptide or a fragment thereof is an altered or mutated MAP3K11 polypeptide or a fragment thereof comprising the alteration or mutation.

The determination of binding may be performed by various techniques, such as by labelling of the test compound, by competition with a labelled reference ligand, etc.

A further object of this invention resides in a method of selecting biologically active compounds, said method comprising contacting in vitro a test compound with a MAP3K11 polypeptide according to the present invention and determining the ability of said test compound to modulate the activity of said MAP3K11 polypeptide. Preferably, said MAP3K11 polypeptide or a fragment thereof is an altered or mutated MAP3K11 polypeptide or a fragment thereof comprising the alteration or mutation.

A further object of this invention resides in a method of selecting biologically active compounds, said method comprising contacting in vitro a test compound with a MAP3K11 gene according to the present invention and determining the ability of said test compound to modulate the expression of said MAP3K11 gene. Preferably, said MAP3K11 gene or a fragment thereof is an altered or mutated MAP3K11 gene or a fragment thereof comprising the alteration or mutation.

In an other embodiment, this invention relates to a method of screening, selecting or identifying active compounds, particularly compounds active on obesity or metabolic

disorders, the method comprising contacting a test compound with a recombinant host cell comprising a reporter construct, said reporter construct comprising a reporter gene under the control of a MAP3K11 gene promoter, and selecting the test compounds that modulate (e.g. stimulate or reduce) expression of the reporter gene. Preferably, said MAP3K11 gene promoter or a fragment thereof is an altered or mutated MAP3K11 gene promoter or a fragment thereof comprising the alteration or mutation.

In a particular embodiment of the methods of screening, the modulation is an inhibition. In an other particular embodiment of the methods of screening, the modulation is an activation.

The above screening assays may be performed in any suitable device, such as plates, tubes, dishes, flasks, etc. Typically, the assay is performed in multi-wells plates. Several test compounds can be assayed in parallel. Furthermore, the test compound may be of various origin, nature and composition. It may be any organic or inorganic substance, such as a lipid, peptide, polypeptide, nucleic acid, small molecule, etc., in isolated or in mixture with other substances. The compounds may be all or part of a combinatorial library of products, for instance.

PHARMACEUTICAL COMPOSITIONS, THERAPY

A further object of this invention is a pharmaceutical composition comprising (i) a MAP3K11 polypeptide or a fragment thereof, a nucleic acid encoding a MAP3K11 polypeptide or a fragment thereof, a vector or a recombinant host cell as described above and (ii) a pharmaceutically acceptable carrier or vehicle.

The invention also relates to a method of treating or preventing obesity or an associated disorder in a subject, the method comprising administering to said subject a functional (e.g., wild-type) MAP3K11 polypeptide or a nucleic acid encoding the same.

An other embodiment of this invention resides in a method of treating or preventing obesity or an associated disorder in a subject, the method comprising administering to said subject a compound that modulates, preferably that activates or mimics, expression or

activity of a MAP3K11 gene or protein according to the present invention. Said compound can be an agonist or an antagonist of MAP3K11, an antisense or a RNAi of MAP3K11, an antibody or a fragment or a derivative thereof specific to a MAP3K11 polypeptide according to the present invention. In a particular embodiment of the method, the modulation is an inhibition. In an other particular embodiment of the method, the modulation is an activation.

The invention also relates, generally, to the use of a functional MAP3K11 polypeptide, a nucleic acid encoding the same, or a compound that modulates expression or activity of a MAP3K11 gene or protein according to the present invention, in the manufacture of a pharmaceutical composition for treating or preventing obesity or an associated metabolic disorder in a subject. Said compound can be an agonist or an antagonist of MAP3K11, an antisense or a RNAi of MAP3K11, an antibody or a fragment or a derivative thereof specific to a MAP3K11 polypeptide according to the present invention. In a particular embodiment of the method, the modulation is an inhibition. In an other particular embodiment of the method, the modulation is an activation.

The present invention demonstrates the correlation between obesity (and related disorders) and the MAP3K11 gene locus. The invention thus provides a novel target of therapeutic intervention. Various approaches can be contemplated to restore or modulate the MAP3K11 activity or function in a subject, particularly those carrying an altered MAP3K11 gene locus. Supplying wild-type function to such subjects is expected to suppress phenotypic expression of obesity and associated disorders in a pathological cell or organism. The supply of such function can be accomplished through gene or protein therapy, or by administering compounds that modulate or mimic MAP3K11 polypeptide activity (e.g., agonists as identified in the above screening assays).

The wild-type MAP3K11 gene or a functional part thereof may be introduced into the cells of the subject in need thereof using a vector as described above. The vector may be a viral vector or a plasmid. The gene may also be introduced as naked DNA. The gene may be provided so as to integrate into the genome of the recipient host' cells, or to remain extra-chromosomal. Integration may occur randomly or at precisely defined sites, such as through homologous recombination. In particular, a functional copy of the MAP3K11 gene may be inserted in replacement of an altered version in a cell, through homologous

recombination. Further techniques include gene gun, liposome-mediated transfection, cationic lipid-mediated transfection, etc. Gene therapy may be accomplished by direct gene injection, or by administering ex vivo prepared genetically modified cells expressing a functional MAP3K11 polypeptide.

Other molecules with MAP3K11 activity (e.g., peptides, drugs, MAP3K11 agonists, or organic compounds) may also be used to restore functional MAP3K11 activity in a subject or to suppress the deleterious phenotype in a cell.

Restoration of functional MAP3K11 gene function in a cell may be used to prevent the development of obesity or metabolic disorders or to reduce progression of said diseases. Such a treatment may suppress the obese phenotype of a cell, particularly those cells carrying a deleterious allele.

Further aspects and advantages of the present invention will be disclosed in the following experimental section, which should be regarded as illustrative and not limiting the scope of the present application.

EXAMPLES

1. Identification of an Obesity susceptibility locus on human chromosome 11

A. Linkage studies

Hager et al. (1998) first identified evidence for linkage with a locus on human chromosome 11 linked to massive human obesity.

Hinney at al. (2002) replicated the linkage to chromosome 11 in an independent genome wide scan. The maximum evidence for linkage (MLS= 1.48) was found between markers D11S903, D11S1313 and D11S1883 at position 44931408 to 69727939. A further study (Price RA, 2001) independently confirmed linkage of this locus in an American population. It is therefore currently safe to conclude that a substantial number of obese families harbour (an) allele(s) predisposing to obesity in a gene on chromosome 11.

B. GenomeHIP platform to identify the chromosome 11 susceptibility gene

As outlined above, the chromosomal interval of the initial linkage findings is huge (39 cM), not allowing a positional cloning approach to identify the obesity susceptibility gene in this region. We applied our GenomeHIP platform to reduce the region to a small interval that would allow rapid identification of the obesity susceptibility gene.

Briefly, the technology consists of forming pairs from the DNA of related individuals. Each DNA is marked with a specific label allowing its identification. Hybrids are then formed between the two DNAs. A particular process (WO00/53802) is then applied that selects all fragments identical-by-descent (IBD) from the two DNAs in a multi step procedure. The remaining IBD enriched DNA is then scored against a BAC clone derived DNA microarray that allows the positioning of the IBD fraction on a chromosome.

The application of this process over many different families results in a matrix of IBD fractions for each pair from each family. Statistical analyses then calculate the minimal IBD regions that are shared between all families tested. Significant results (p-values) are evidence for linkage of the positive region with the trait of interest (here obesity). The linked interval can be delimited by the two most distant clones showing significant p-values.

In the present study, 89 families of German origin (117 independent sib-pairs) concordant for massive obesity (as defined by a body mass index > 90%ile) were submitted to the GenomeHIP process. The resulting IBD enriched DNA fractions were then labeled with Cy5 fluorescent dyes and hybridised against a DNA array consisting of 9946 chromosome 11 derived human BAC clones covering the linkage region completely (positions 45451827 to 84002801). Non-selected DNA labelled with Cy3 was used to normalize the signal values and compute ratios for each clone. Clustering of the ratio results was then performed to determine the IBD status for each clone and pair.

By applying this procedure, one BAC clones (RP11-9K14) spanning approximately 156 kilo bases in the region on chromosome 11 (bases 66954126 to 67110369) was identified, that showed significant evidence for linkage to obesity $p < 2.5 \times 10^{-5}$).

C. Identification of an obesity susceptibility gene on chromosome 11

By screening the aforementioned 156 kilo bases in the linked chromosomal region, we identified the ***mitogen activated protein kinase kinase kinase*** (MAP3K11) gene as a candidate for obesity and related phenotypes. This gene is indeed present in the critical interval, with evidence for linkage delimited by the clone outlined above.

MAP3K11 gene encodes a predicted 847-amino acid polypeptide (mRNA 3.5 kb) and spreads over 15.5 kb of genomic sequence. The protein encoded by the gene is a member of he serine/threonine mixed lineage kinase family and contains a SH3 and leucine zipper domains. The kinase can directly phosphorylate and activate IkappaB kinase alpha and beta and has been found to be involved in the transcription activity of NF-kappaB.

Most importantly this kinase preferentially activates JNK1 (MAPK8) and functions as a positive regulator of the JNK signalling pathway.

It has recently been shown, that JNK1 activity is abnormally elevated in obesity (J. Hirosumi et al., Nature 420:333 – 336, 2002) and that JNK is a crucial mediator of obesity and insulin resistance.

Furthermore it was shown that an absence of JNK1 activity resulted in decreased adiposity. MAP3K11 has a significance influence on JNK activity and allelic forms of MAP3K11 may therefore be responsible for this profound change of JNK1 activity as seen in obesity.

Taken together, the linkage results provided in the present application, identifying the human MAP3K11 gene in the critical interval of genetic alterations linked to obesity on chromosome 11, with its involvement in the JNK signalling pathways, we conclude that alterations (e.g., mutations and/or polymorphisms) in the MAP3K11 gene or its regulatory sequences may contribute to the development of human obesity and represent a novel target for diagnosis or therapeutic intervention.

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